Extreme occupational heat exposure is associated with elevated haematological and inflammatory markers in Fire Service Instructors

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Title:

Extreme occupational heat exposure is associated with elevated haematological and inflammatory markers in Fire Service Instructors

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Inflammation in Fire Instructors

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NEW FINDINGS

What is the central question of this study?

Fire service instructors are frequently exposed to live fire scenarios, representing the most extreme chronic occupational heat exposure. These individuals report a series of unique health issues. This study seeks to identify if the number of exposures completed are associated with inflammatory and immunological markers and symptoms of ill health.

What is the main finding and its importance?

Fire service instructors exhibit greater levels of inflammatory markers in comparison to firefighters. Fire exposure numbers are positively related with the prevalence of ill health and inflammation. Implementation of a proposed 9 exposure limit per month may be appropriate to minimise health issues.

ABSTRACT

Fire Service Instructors (FSI) experience ~10 times more fire exposures than firefighters (FF), the increased physiological stress from this potentially puts them at risk of ill health and future cardiac events. This study aimed to establish whether FSI exhibit elevated biomarkers associated with cardiac event risk, identify if FSI experience systemic inflammation linked to fire exposure frequencies and evaluate a proposed exposure limit of 9 per month. Blood samples were collected from 110 Fire Service personnel (age: 44 ± 7 yrs; height: 178.1 ± 7.1 cm; body mass: 84.3 ± 12.0 kg, FSI n = 53, FF n = 57) for biomarker analysis. Work history details were collected from all participants. Participants with biomarker concentrations above healthy reference ranges were classified as "at risk". Neutrophil/lymphocyte ratio, platelet count, cardiac troponin T, interleukin-6 (IL-6), interleukin-1β (IL-1β), immunoglobulin G (IgG) and C reactive protein (CRP) were greater in FSI than FF ($p<0.05$). Multiple regression analysis revealed 18.8% of IL-6, 24.9% of IL-1β, 29.2% of CRP, and 10.9% of IgG variance could be explained by number of heat exposures per month. Odds ratios revealed that those above the 9 per month exposure limit were 6-12 times more likely to be classified as "at risk" and were 16 times more likely to experience symptoms of ill health. Increased cytokine levels suggest FSI experience systemic inflammation, which is related to symptoms of ill health. We propose that an exposure limit could reduce the prevalence of these biomarker risk factors and ill health.

INTRODUCTION

Within the UK Fire and Rescue Service, Fire Service Instructors (FSI) are responsible for the training of newly recruited and operational firefighters (FF). FSI regularly experience fire scenarios (commonly referred to as wears) to enable them to deliver training on breathing apparatus use, search and rescue techniques and fire behaviour. On average FSI report completing ~13 exposures a month, compared to ~1 fire incident attended by FF in the same period (Watkins, Hayes, Watt, & Richardson, 2018a). During exposures FSI typically experience maximum core temperatures of 37.9 – 38.5°C, with maximum heart rates of 134 – 147 $b.min^{-1}$ (Eglin, Coles, & Tipton, 2004; Watkins, Hayes, Watt, & Richardson, 2019b; Watt et al., 2016); indicating that these individuals undergo a moderate level of physiological strain on a regular basis.

A recent survey of FSI highlighted that 41% reported experiencing new symptoms of ill health since starting their career as a FSI (Watkins et al., 2018a). Symptoms included: severe fatigue, broken sleep, mood swings and headaches. Smith et al. (2000) hypothesised that symptoms such as these may be associated with chronic systemic inflammation when occurring in relation to repeated high volume physical strain without sufficient rest. However, it is difficult to ascertain whether inflammation is the cause of these symptoms, as there are limited possibilities to study individuals who experience high levels of strain over long periods of time (Carfagno & Hendrix, 2014). Previous assessment of military recruits during training courses have identified symptoms, including sleep disturbances, fatigue and confusion, that coincide with elevated interleukin-6 (IL-6), tumour necrosis factor alpha (TNFα) and C reactive protein (CRP) (Booth, Probert, Forbes-Ewan, & Coad, 2006; Gomez-Merino, Chennaoui, Burnat, Drogou, & Guezennec, 2003). The presence of elevated cytokine IL-6 has also been reported in FSI in comparison to non-fire exposed controls (Watt et al., 2016; Watkins, Hayes, Watt, & Richardson, 2019a;), although only a small sample of the FSI population have been assessed.

Inflammation is linked to the presence of atherosclerosis and cardiovascular event risk (Moriya, 2019). Numerous haematological factors such as IL-6, CRP, and platelet counts (PLT) are involved in the inflammatory process and are also predictors of cardiovascular event risk (Pearson et al., 2003; Ridker, Rifai, Stampfer, & Hennekens, 2000; Sharma & Berger, 2011). FSI, along with FF, have an increased risk of a cardiovascular event following a live fire (Kales et al. 2007), it is also the leading cause of death within FF (Fahy, Leblanc, & Molis, 2015). Currently, research has focused on the immediate impact that live fire has to the risk of cardiac events (Fahs et al., 2011; Fernhall, Fahs, Horn, Rowland, & Smith, 2012; Hunter et al., 2017). Findings by Watt et al (2016) and Watkins et al., (2019b) highlight that FSI exhibit increases in PLT, IL-6, and cardiac troponin T (cTnT), a marker of cardiac muscle damage, after a training-based fire exposure, however the long term implications of this acute response are not yet fully understood. It can be theorised that if a live fire causes an increased risk, with minimal recovery time between fires, this risk may be elevated further by subsequent exposures if nothing is done to reduce the risk between events. It is therefore of importance to establish whether the number of fire exposures experienced is linked to an elevation in haematological markers associated with cardiovascular risk on a daily basis.

The frequency of wear completion by FSI may be instrumental in the scale and time course of inflammation development. A survey of FSI highlighted that there is currently no evidenced based guidance on the number of wears a FSI should remain below in any given period (Watkins et al., 2018a). Although 55% of FSI did have a limit set by management, they varied across services from 2 - 10 per week. In the USA instructors have been reported to complete 3 -5 live fires per day over a period of several weeks or months (Fent et al., 2019). The most commonly reported limit to exposure numbers in the UK was 9 per month, which is not a formal policy, rather one proposed by the UK Chief Fire Officers' Association (2015). Concerns regarding the number of wears performed was also a key theme when FSI offered their opinions about their working practices, with 35% of FSI indicating they felt that they completed too many wears. Being able to establish a suitable wear limit that might minimise the risk of fatigue symptoms occurring and reduce cardiac event risk factors, would enable evidence based guidelines for FSI across the world. Such information could have the potential to reduce FSI cardiac events risks and symptoms of ill health and prolong time spent in the career.

This study aimed to establish whether FSI exhibit an altered baseline immunological level compared to FF. In addition, it aimed to identify if altered immunological levels were related to the number of wears completed by FSI. The final aim of the study was to evaluate a proposed limit of 9 wears a month, to establish if completing wear numbers above or below this number was associated with immunological markers above upper reference ranges and reports of ill health. There are therefore four hypotheses within this study: (1) FSI will exhibit greater IL-6, CRP, cTnT and platelet volume and counts than FF, (2) increased fire exposure numbers will be correlated with elevated levels of cytokines, (3) those above the 9 wear limit will exhibit cytokine levels above the reference limits, and (4) there will be an association between immunological markers and reports of ill health.

METHODS

Ethical Approval

One hundred and ten individuals (age: 44 ± 7 yrs; height: 178.1 ± 7.1 cm; body mass: 84.3 ± 12.0 kg) were recruited from Fire and Rescue Services across the UK to participate in the study. Of the 110 recruits, 53 were FSI (age: 45 ± 8 yrs; height: 176.6 ± 8.1 cm; body mass: 84.1 ± 8.1 kg) and 57 were FF (age: 44 ± 7 yrs; height: 179.5 ± 5.8 cm; body mass: 84.4 ± 10.1 kg). Of the FSI, 47 (89%) were male and 6 (11%) were female. Of the FF, 55 (96%) were male and 2 (4%) were female. Independent fitness testing was not conducted as part of this research study, however all participants were operational personnel and therefore were required to meet national fitness standards. Fitness standards in the UK Fire and Rescue Service state that operational personnel should maintain a minimum maximal volume of oxygen uptake of 42 $mL.kg^{-1}.min^{-1}$ (Stevenson, Wilsher, & Sykes, 2009), which is assessed in Service by trained staff. All participants took part in physical activity 2 – 3 times a week, with 32% reporting activity 4 – 5 times a week and 5% performing physical activity >5 times a week. Participants gave informed written consent and completed a medical questionnaire prior to taking part. Participants were required to abstain from caffeine and exercise for 12 hours prior to the study commencing, they were also asked to avoid alcohol for 24 hours prior to taking part. Adherence to these parameters was checked via a questionnaire. The study was approved by the University of Brighton Research Ethics Committee (reference number: ESREGC/09/15) and conformed to the Declaration of Helsinki (2013), except for registration in a database.

Experimental Design

Participants provided a single venous blood sample and working history details on one occasion. This was collected at the Welkin Laboratories, University of Brighton or on location at Fire Stations and Fire Training Centres. Samples were collected from all FSI between 8:00 - 10:00am. Samples were collected from FF between 8:00 - 10:00am or from 5:00 - 7:00pm, depending on their availability due to work requirements and rotas. All samples were collected prior to any heat exposure that day and a minimum of 12 hours post the last fire exposure.

Venous Blood Collection

A 10mL venous blood sample was taken at rest from the anti-cubital fossa by a trained phlebotomist. All samples were analysed within 2hr of collection for complete blood counts using an automated haematology analyser (XT2000i, Sysmex, UK) and then centrifuged at 4,500 rpm for 10 min at 4°C for plasma separation. Plasma was stored in a -86°C freezer for subsequent ELISA analyses of: IL-6, TNFα, IL1-β, CRP (R & D Systems, Minneapolis, USA) and IgG (eBioscience, Thermo Fischer Scientific, Massachusetts, USA). The inter-assay coefficient of variation (CV%) for each ELISA was: 5.5% IL-6, 8.7% TNFα, 11.5% IL-1β, CRP 11.7% and IgG 6.0%. Plasma samples were also analysed for high sensitivity cTnT using an electrochemiluminescence assay [Roche Modular E170 (fifth generation), Basel, Switzerland], which had a blank of 3 ng.L^{-1} and CV at the upper reference limit (14 ng.L^{-1}) of < 8% (Westermann, Neumann, Sörensen, & Blankenberg, 2017). Upper reference limits were identified for all haematological variables, with limits for variables measured via ELISA analysis increased by the corresponding inter-assay CV. IL-6 and CRP limits represent increased risk of a cardiovascular event. The upper quartile for IL-6 of 2.28pg.mL^{-1} was selected as Ridker et al., (2000) identified that those above this value have a 2.3 times higher relative risk of a future myocardial infarction than those in the lowest quartile. For CRP a value of >3 mg.L^{-1} was chosen as it represents the high risk category, with a 2 fold increase in relative risk of cardiovascular disease compared to those with < 1.0 mg.L^{-1} (Pearson et al., 2003). All other variable limits represent healthy upper reference ranges. Limits and their sources are detailed in Table 2.

Participant Details and Working History

Participants were asked if in the previous month they had suffered from symptoms of ill health, identified by Watkins et al., (2018a) (fatigue, broken sleep, heavy sweating, heart palpitations, mood swings, coughing, and breathing problems) or any other illnesses. Participants were also asked to provide a brief work history. Details of their time in the Fire and Rescue Service, if they were currently a FSI, and the time they had spent in their current role were collected. Participants also reported how many fire exposures (in either a training or operational capacity) they had completed in the previous week and in the last month.

Statistical Analysis

Data were analysed using IBM SPSS Statistics 22 and presented as mean ± SD, unless otherwise stated. Data were tested for normality and sphericity. Differences in haematological variables between FSI and FF were assessed via independent samples t-tests, or Mann-Whitney U tests when data were not normally distributed. Cohens d_s effect sizes are presented for t-test comparisons (Lakens, 2013) whilst r effect sizes are given for Mann-Whitney U tests (Field et al., 2009), effect sizes are interpreted as recommended by Cohen (1988). Pearson's chi-squared analysis was conducted to establish if there was an association between occupational group (FSI vs FF) and occurrence of symptoms of ill health (YES vs NO), with subsequent odds ratios conducted where significant associations occurred.

Multiple regression analysis was conducted to identify the relationship between work history and demographic variables (BMI, age, time in service, number of wears completed per week, number of wears completed per month) with haematological dependent variables. Where dependent variables did not meet normality assumptions bootstrap re-sampling was conducted. Where significant regression models were identified, regression coefficient statistical significance was interrogated for each predictor variable. Regression analysis was then rerun with only significant predictor variables to define the model.

Haematological markers identified as predicted from exposure numbers had data classified as either above ("at risk") or below ("healthy") upper reference range values (see Table 2). To evaluate the effectiveness of the current FSI proposed limit of 9 wears a month, Pearson's chi squared analysis was conducted to identify associations between those performing ≤ 9 or > 9 exposures a month and those in the "at risk" or "healthy" groups. Pearson's chi squared analysis was also conducted to determine if reference range groups ("at risk" vs "healthy") were associated with ill health symptoms, with follow up odds ratios conducted for significant associations. An a priori power analysis was performed using previously reported differences in IL-6 between FSI and a control group (Watkins et al., 2019a), on the basis of the effect size (Cohen's d = 1.11) associated with those differences and a statistical power of 80% a minimum of 28 participants were required. Due to the limited previous data available for the association of the specific symptoms of ill health with elevated cytokine levels, an a priori test based on a chi squared analysis with a medium effect (w = 0.3) and 80% power was conducted, identifying that a minimum of 88 participants were required. Significance level was set at $p < 0.05$.

RESULTS

Differences between FSI and FF

FSI and FF had similar demographic details (Table 1). FSI had completed a greater number of fire exposures in the previous week and month ($p < 0.001$) in comparison to FF (Table 1). The maximum number of exposures completed in a week and month by FSI were 8 and 20, respectively. In total 37 (69%) FSI were within the suggested limit of ≤ 9 wears per month, whilst 16 were not. No FF completed > 3 exposures per month.

Reports of ill health were made by 3 (5%) FF and 16 (30%) FSI. FF who reported illness all reported colds. Of the FSI who reported illness, 10 (19%) had severe fatigue, 8 (15%) had a cold, 8 (15%) had broken sleep, 4 (8%) suffered from a cough, 4 (8%) had heavy sweating, 2 (4%) had mood swings, 1 (2%) suffered from heart palpitations and 1 (2%) had sinusitis. Pearson's chi squared analysis revealed a significant association between group and presence of illness (χ^2 (1) =11.941, p = 0.001), with FSI 7.78 (95% CI 2.12 - 28.62) times more likely to have a symptom of ill health than FF.

Analysis of CBC between FSI and FF revealed increased levels of NEUT (p = 0.029), PLT ($p < 0.001$), BASO (p = 0.003), and neutrophil lymphocyte ratio (NLR) (p = 0.044) in FSI compared to FF. FF had increased levels of

EO ($p = 0.032$) than FSI. Analysis of other haematological variables revealed increases in cTnT ($p < 0.001$), IL-6 ($p = 0.002$), IL-1β ($p = 0.006$), CRP ($p = 0.005$), and IgG ($p < 0.001$) in comparison to FF. See Table 2 for details of haematological levels.

Relationship between Deterministic Variables and Haematological Markers

Multiple regression analysis of haematological variables revealed significant models for IL-6, IL-1β, CRP and IgG only ($p < 0.05$). For these four haematological markers, age, time in service, and week exposure number were not predictor variables ($p > 0.05$). Following remodeling with only significant predictors, month exposure number explained 18.8% of IL-6 variance, 24.9% of IL-1β variance, 29.2% of CRP variance, and 10.9% of IgG variance (see Figure 1). BMI also explained an additional 3% of CRP variance.

"At Risk" Reference Values Associated with Wear Limits and Ill Health

Haematological variables related to monthly wear numbers (IL-6, IL-1β, CRP and IgG) were grouped according to the upper reference range criteria. Those below the reference limit were classified as the "healthy" group, whilst those above were classified as the "at risk" group. The current suggested monthly wear limit of 9 wears was associated with groupings for IL-6, IL-1β, CRP and IgG, see Table 3 for the association statistics and odds ratios. The 9 wear monthly limit was also associated with the presence of symptoms of ill health (χ^2 (1) = 26.803, $p < 0.001$). Those above the wear limit were 15.74 times (95% CI 2.26 - 32.80) more likely to suffer symptoms of ill health than those below the limit. The reference limit groupings for IL-6, IL-1β, CRP and IgG were also associated with the presence of symptoms of ill health, see Table 4 for the association statistics and odds ratios.

DISCUSSION

This study aimed to evaluate the concentrations of immunological markers related to health problems in FSI and FF in association with exposure history and the proposed 9 wears per month limit. Differences in numerous immunological markers were noted between FSI and FF, with FSI having greater levels of biomarkers associated with future cardiac events, such as NLR, IL-6 and CRP. In addition, monthly wear numbers were positively related to IL-6, IL-1β, CRP and IgG concentrations. The current proposal of a 9 wear limit may be reasonable, with those above the limit more likely to be in "at risk" groups for raised IL-6, IL-1β, CRP and IgG. Moreover, those in "at risk" groups were more likely to experience symptoms of ill health.

Of the FSI involved in this study, 30% reported symptoms of ill health, compared to 5% of FF. The occurrences reported are lower than the 41% of FSI and 21% of FF who reported ill health in a previous survey of fire service personnel (Watkins et al., 2018a). However, that survey referred to frequent symptoms since the beginning of their job role, whereas this study gathered information of symptoms experienced within the previous month. The type of symptoms reported in this study are however similar to that previously referred to, reaffirming the prevalence of a set of symptoms in the FSI population different from their FF counterparts. Comparison of FSI and FF resting haematological variables revealed that NEUT, PLT, BASO, NLR, cTnT, IL-6, IL-1β, CRP, and IgG were all greater in FSI.

Acute Inflammatory and Haematological Changes with Heat Exposure

Numerous previous studies offer an assessment of haematological and inflammatory responses to heat exposure. Following live fire exposures an increase of 19 – 85% in WBC and 32 – 54% in NEUT have typically been reported, with extended durations and greater temperatures resulting in larger increases when comparing studies (Smith, Petruzzello, Chludzinski, Reed, & Woods, 2005; Smith et al., 2011; Watkins et al., 2019b; Watt et al., 2016). Inflammatory markers such as IL-6 have also been noted to increase by 25 – 27% with live fire exposure (Walker et al., 2015; Watkins et al., 2019b; Watt et al., 2016).

The elevation of these markers has been postulated to be stimulated through numerous mechanisms. Sympathoadrenal activation, as a result of physiological strain, increases circulating catecholamines, which can reduce the interaction between leukocytes and endothelial cells and consequently cause the demargination of leukocytes from the vasculature (Shephard, 2003; Smith et al., 2005; Walsh & Whitham, 2006). Tissue damage can stimulate an inflammatory response, with endothelial cells releasing IL-6 and subsequently stimulating the release other inflammatory markers, such as C-reactive protein, and leukocyte transmigration as part of the acute phase response reaction (Bruunsgaard et al., 1997). However, it is now well documented that muscles release IL-6 during exercise and periods of elevated temperature without the presence of tissue damage (Fischer, 2006; Petersen & Pedersen, 2005; Welc et al., 2012). Endotoxin release from the gut during exercise with heat stress is also proposed as a stimulant for inflammation (Snipe, Khoo, Kitic, Gibson, & Costa, 2018; Starkie, Hargreaves, Rolland, & Febbraio, 2005). Research suggests that whilst these haematological markers may remain altered compared to baseline in the hours immediately following exposure, by 24 hours they typically have returned to resting levels (Walker et al., 2015). Consequently additional exposures with minimal recovery could result in the baseline elevations reported in this study.

Cardiovascular Event Risk

An increased NLR represents neutrophilia, also indicated by the noted increase in NEUT, in combination with lymphocytopaenia, and is an indicator of systemic inflammation (Guthrie et al., 2013). NLR is known to increase following acute exercise, remaining elevated but returning towards normal values 6 hrs post exercise (Nieman, 1998; Nieman, 2000). Neutrophils are involved in all stages of atherosclerosis, they increase the expression of adhesion molecules, limit vasodilation, and can lead to atherosclerotic plaque instability, making the plaque prone to rupture (Soehnlein, 2012). In contrast, regulatory T cell lymphocytes are involved in the inhibition of atherosclerosis by regulating the inflammatory response and therefore low lymphocyte counts represent a poorly regulated immune response (Shah et al., 2014). Consequently, the combined ratio of these two white blood cells acts as an independent predictor of cardiac mortality in patients with coronary artery disease (Papa et al., 2008), those with acute decompensated heart failure (Uthamalingam et al., 2011), patients with myocardial infarctions (Bhat et al., 2013), and in a healthy general population (Shah et al., 2014). Within a healthy population, higher NLR is associated with a greater incidence of coronary heart disease related deaths, with an NLR >4.5 associated with 11% of deaths compared to 3.2% of deaths with an NLR of 1.5 – 3.0 and 2.4% of deaths in those with an NLR of <1.5 (Shah et al., 2014). The upper range of NLR in a healthy population is 3.53, however an NLR >4.5 is associated with a hazard ratio for future cardiac events of 2.68 (Forget et al., 2017; Shah et al., 2014). Fest et al., (2019) also states that being in the 3rd (1.60 – 1.91), 4th (1.92 – 2.41) and 5th (>2.41) quartiles for NLR increased all cause mortality in comparison to the 1st quartile (<1.30). Whilst the exact values attributed to quartiles and high NLR are study dependent, FSI in this study exhibit a greater NLR (2.12) than FF (1.83), suggesting that FSI may be at a greater risk of a cardiovascular event than FF.

Elevated cTnT in FSI indicates possible low level myocardial damage. Baseline resting measurements of cTnT from healthy populations indicate typical levels of < 10 $ng.L^{-1}$ in marathon runners (Neilan et al., 2006; Richardson et al., 2018) and 6.2 ± 2.2 $ng.L^{-1}$ in elite floorball players (Wedin & Henriksson, 2015). This suggests that cTnT exhibited by FF and FSI are both below the healthy upper limit (< 14 $ng.L^{-1}$) and within the range of other healthy active population groups. Increased resting levels of cTnT in healthy individuals are related to left ventricular wall thickening and left ventricular systolic dysfunction, in addition to all cardiovascular mortality (de Lemos et al., 2010). With each tertile increase of cTnT from non-detectable to > 14 $ng.L^{-1}$, the hazard ratio of cardiovascular mortality increases. Those with cTnT between 3 - 4.4 $ng.L^{-1}$ have a hazard ratio of 1.6 (95% CI 0.5 - 4.9), a cTnT of 4.4 - 6.6 $ng.L^{-1}$ suggests a ratio of 2.4 (95% CI 0.9 - 6.1), and individuals with cTnT 6.6 - 14 $ng.L^{-1}$ the hazard ratio of cardiovascular mortality is 4.6 (95% CI 2.1 - 10.0) (de Lemos et al., 2010). FF consequently have a lower hazard ratio of cardiovascular mortality than FSI, with FF exhibiting 3.00 ± 1.32 $ng.L^{-1}$ and therefore either falling in undetectable levels or within the first detectable tertile of 3 - 4.4 $ng.L^{-1}$, compared to FSI who exhibited 4.41 ± 2.68 $ng.L^{-1}$ giving the average FSI a hazard ratio of 2.4.

MPV is also associated with incidence of myocardial infarction and coronary artery disease (Klovaite, Benn, Yazdanyar, & Nordestgaard, 2011; Sansanayudh et al., 2014). However, MPV displayed no differences between FSI and FF and although FSI PLT (234 ± 79 $x10^9.L^{-1}$) was greater than FF PLT (190 ± 35 $x10^9.L^{-1}$), it remained below the upper reference range. This is similar to the PLT noted in previous FF studies of 264 ± 53 $x10^9.L^{-1}$ (Smith et al., 2011), 257 ± 62 $x10^9.L^{-1}$ (Smith et al., 2014) and 241 ± 11 $x10^9.L^{-1}$ (Hunter et al., 2017), and resting PLT previously reported in FSI (209 ± 43 $x10^9.L^{-1}$) (Watkins et al., 2019b). Consequently demonstrating that whilst elevated PLT has been noted post fire exposure (Smith et al., 2011; Hunter et al 2017; (Watkins et al., 2019b), this increase is transient with minimal effect on daily baseline levels.

A key finding of this study is the significantly elevated levels of inflammatory cytokines IL-6, IL-1β and acute phase protein CRP, in FSI. IL-6 is involved in the increase of cell adhesion molecules, platelet reactivity and CRP release, and therefore may be involved in atherosclerosis formation (Lindmark, Diderholm, Wallentin, & Siegbahn, 2001; Schuett, Luchtefeld, Grothusen, Grote, & Schieffer, 2009). FSI exhibited a mean IL-6 of 1.66 ± 2.29 $pg.mL^{-1}$, in the third quartile of values reported in apparently healthy men (1.47 – 2.28 $pg.mL^{-1}$), such concentrations have been linked to a 2.8 times higher risk of myocardial infarction (Ridker et al., 2000). IL-6 levels in FSI reported in this study are not as great as those reported at rest by Watt et al (2016) (7.4 – 17.0 $pg.mL^{-1}$). However, this present study included FSI with an average of 6 ± 5 exposures a month, whereas FSI who participated in the study conducted by Watt et al (2016) had completed 15 wears in a 4 week period. The participants therefore experienced a greater number of exposures than the average experienced by the FSI involved in this study, further supporting the positive correlation identified between exposure number and IL-6 values.

IL-1β is also involved in increasing the expression of adhesion molecules, inducing procoagulant activity, and the stimulation of CRP synthesis and release (Jialal, Devaraj, & Venugopal, 2004; Ridker, Thuren, Zalewski, & Libby, 2011). Although IL-1β has not yet been established as a predictor of cardiovascular events with set classifications of risk, perhaps due to the variability in its measurement in plasma (Ridker et al., 2011), gene polymorphisms causing increased IL-1β are associated with risk of coronary artery disease and cardiovascular events (Tsimikas et al., 2014). There is some effort to target IL-1β for atheroprotective interventions and there is some underpinning evidence for its involvement in raised risk (Ridker, 2016). This current study is the first study to identify elevated IL-1β levels in FSI.

CRP has also been detected in atherosclerotic lesions (Yasojima, Schwab, McGeer, & McGeer, 2001) and elevated levels have been reported to correlate with increased adhesion molecules and lower endothelium-dependent vasodilatory responses (Fichtlscherer et al., 2000; Jialal et al., 2004), although it is unlikely CRP is causal in these endothelial changes (Danesh & Pepys, 2009; Taylor, Giddings, & Van Den Berg, 2005). Whilst the mean CRP exhibited by FSI was 1.62 ± 2.40 mg.L^{-1}, the variation in levels was high (as demonstrated by the large SD). Of the FSI, 26.4% of individuals displayed CRP > 3.0 mg.L^{-1} in comparison to just 3.5% FF. CRP > 3.0 mg.L^{-1} indicates a 2 fold increase in relative risk of cardiovascular disease compared to those with CRP < 1.0 mg.L^{-1} (Pearson et al., 2003). The elevated presence of IL-6, IL-1β, and CRP therefore demonstrates FSI experience chronic systemic inflammation and are at an increased risk of cardiovascular events.

Wear Limits

The measured concentration of IL-6, IL-1β, CRP and IgG were related to the number of wears that had been completed in the previous month, with 11 - 29% of variance in these markers explained by wear numbers. These findings support the theory that performing a greater number of fire exposures is associated with increased cytokine levels. The wear limit informally proposed and followed by some training centres is 9 wears per month (Watkins et al., 2018a). The evaluation of this limit indicates that those above the limit are more likely to be in the "at risk" group for IL-6 (OR = 6.27), IL-1β (OR = 7.00), CRP (OR = 12.43) and IgG (OR = 7.55). This suggests that those FSI conducting a greater number of wears may be at an increased risk of a future cardiovascular event. Moreover, our data suggests that individuals completing > 9 wears a month are 15.74 times more likely to suffer symptoms of ill health than those below the limit.

Health Symptoms

The "at risk" groups for IL-6, IL-1β, CRP, and IgG were also associated with an increased likelihood of experiencing symptoms of ill health, such as fatigue, sleep disturbances, headaches, colds and flu like illnesses (IL-6 OR = 5.63, IL-1β OR = 3.67, CRP OR = 6.05, IgG OR = 6.45). These symptoms are comparable to "sickness behaviours", such as depression, fatigue and sleep disturbances, which are a consequence of the ability of inflammatory cytokines to effect central nervous system function. This may occur through numerous pathways, including: the transportation of cytokines across the blood brain barrier via cytokine receptors, stimulation of afferent nerves such as the vagus nerve and entering the brain via volume diffuse at circumventricular organs (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Johnson, 2002). Within both a military and sporting environment similar findings have been reported, with sleep disturbances, depressed mood and fatigue occurring with elevated levels of IL-1β, TNFα, CRP and IL-6 following repeated high levels of physiological strain (Booth et al., 2006; Gomez-Merino et al., 2003). Consequently, this study indicates that the symptoms reported by FSI may be related to systemic inflammation.

Cold and cough symptoms reported by FSI also suggest the possibility that immune function may be altered; it has previously been proposed that following periods of high physical strain humoral immunity is up-regulated and consequently cellular immunity is suppressed, increasing the risk of viral or bacterial infection (Buyukyazi et al., 2004; Smith, 2004). An increase in humoral immunity may also be related to an increase in allergies and immunoglobulins (Smith, 2004). IgG was elevated in FSI (1649 ± 865 $mg.dL^{-1}$) in comparison to FF (835 ± 695 $mg.dL^{-1}$), possibly indicating increased humoral immunity and inflammation. However, the balance between cellular and humoral immunity is also carefully controlled by the regulation of specific cytokines. IL-2, IL-12 and interferon gamma (IFNγ) are crucial in the development of Th1 cells that are instrumental in cellular immunity, and IL-4, IL-5, IL-10, IL-6, and TNFα being involved in the development of Th2 cells that are involved in humoral immunity (Kidd, 2003; Lucille Lakier Smith, 2003). Whilst the elevated IL-6 in FSI could support the possible increase in humoral immunity, a larger array of cytokines and other biomarkers should be investigated in future studies to further understand the balance between humoral and cellular immunity in FSI.

Limitations

Unlike IL-6, IL-1β, CRP and IgG some haematological variables that were greater in FSI than FF were not statistically associated with number of wears completed, or with any other demographic or work history details. In addition, not all variation in IL-6, IL-1β, CRP, and IgG was explained by monthly exposure numbers. It is reasonable to suggest that there are many other factors that were outside of the scope of this specific study that may have influenced these variables, such as the overall thermal load experienced from the wears completed, the type of wears completed (Watkins et al., 2019b), level of smoke/particulate exposure, stress levels of participants, and FSI use of hydration guidance, clothing (Watkins & Richardson, 2017) and pre/post cooling interventions (Watkins, Hayes, Watt, & Richardson, 2018b) for each wear performed. Stress, anxiety and depression have also previously been associated with systemic inflammation (Majd, Saunders, & Engeland, 2019; Renna, O'Toole, Spaeth, Lekander, & Mennin, 2018), but were not measured in this study. It is unknown if levels of these psychological conditions differ between FF and FSI. Future research should further explore the influence that other factors may have on systemic inflammation in FSI.

Participants who reported themselves as FSI were classified as such, regardless of wear exposure number, to ensure the data represented the FSI population. This resulted in two FSI with no wear completions in the previous month being included in the FSI group, despite them having a lower monthly wear number than the FF group mean. Due to the logistical difficulty of accessing this population and cost of blood sample analysis this study offers only a singular snapshot of FSI and FF immunological status. Presence of symptoms of ill health were also subjective and not verified by a medical professional. Furthermore, there are many risk factors of cardiovascular events, of which not all were collected in this study. The duration between wears was also not detailed as part of this study and should be included in future research. Overall, it is suggested that long term monitoring should be conducted to establish if any additional extraneous variables are associated with haematological values.

CONCLUSION

In conclusion, FSI specifically need to be targeted for interventions to help reduce the occurrence of elevated haematological measures, as findings from this study suggest they are at an increased risk of a future cardiovascular event and symptoms of ill health compared to FF. Moreover, increased levels of IL-6, IL-1β, CRP, and IgG are associated with the number of wears FSI complete in a month. The current suggestion of a 9 wear per month limit seems a reasonable guidance, as those above the limit are 15.74 times more likely to experience symptoms of ill health. Future research should investigate the impact that interventions designed to reduce the thermal load from frequent fire exposures have on the chronic systemic inflammation prevalent in FSI.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Blood samples were analysed at the Centre for Sport, Exercise & Life Sciences, Coventry University and the Environmental Extremes Laboratory, University of Brighton. EW, MH, PW and AJ were involved in the conception and design of the work. EW, MH, PW, DR and AJ contributed towards the acquisition, analysis and interpretation of the data. EW, MH, PW, DR and AJ drafted the work or revised it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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TABLES

Table 1 Demographic and work history details for firefighters and fire service instructors. [a] denotes values displayed are median ± IQR. * denotes a difference between the two groups, $p < 0.05$.

	Fire Service Instructors	**Firefighters**
Age (yrs)	45 ± 8	44 ± 7
Body mass (kg)	84.1± 13.8	84.4 ± 10.1
BMI (kg.m^2)	26.5 ± 5.1 [a]	25.4 ± 3.5 [a]
Time in Service (yrs)	19 ± 8	17 ± 7
Time in role (yrs)	3 ± 8 [a] *	10 ± 10 [a]
Weekly Exposures	1 ± 3 [a] *	0 ± 0 [a]
Monthly Exposures	5 ± 8 [a] *	1 ± 1 [a]

Table 2 Resting levels of haematological variables in FSI and FF. [a] denotes values displayed are median ± IQR. Effect size is given as d_s, unless the variable is presented as median ± IQR, whereby the corresponding effect size is r. * denotes a difference between groups, $p < 0.05$.† denotes median value is greater than upper reference value. Upper limit for WBC, RVC, HGB, HCT, NEUT, LYMPH, MONO, EO and BASO from Bain et al. (2011), PLT and MPV from Briggs et al. (2007), NLR from Forget et al. (2017), cTnT from Zhelev et al. (2015), IL-6 from Ridker et al. (2000), TNFα from Todd et al. (2013), IL-1β from Di Iorio et al. (2003) and La Fratta et al. (2018), CRP from Pearson et al. (2003) and IgG from Fuggle (2017).

	Fire Service Instructors	**Firefighters**	**Effect Size**	**Upper Reference Limit**
WBC ($x10^9.L^{-1}$)	6.56 ± 2.10 [a]	6.47 ± 1.73 [a]	0.09	10.0
PLT ($x10^9.L^{-1}$)	234 ± 79 [a] *	190 ± 35 [a]	0.49	400
MPV (fL)	10.0 ± 0.9	10.3 ± 0.7	0.37	11.2
NEUT ($x10^9.L^{-1}$)	3.85 ± 1.20 [a] *	3.54 ± 1.18 [a]	0.18	7.0
LYMPH ($x10^9.L^{-1}$)	1.97 ± 0.52	1.97 ± 0.52	0	3.0
MONO ($x10^9.L^{-1}$)	0.59 ± 0.26 [a]	0.57 ± 0.19 [a]	0.01	1.0
EO ($x10^9.L^{-1}$)	0.13 ± 0.15 [a] *	0.18 ± 0.13 [a]	0.20	0.5
BASO ($x10^9.L^{-1}$)	0.05 ± 0.05 [a] *	0.03 ± 0.03 [a]	0.28	0.1
NLR	2.12 ± 0.84*	1.83 ± 0.63	0.19	3.53
cTNT ($ng.L^{-1}$)	4.41 ± 2.68 [a] *	3.00 ± 1.32 [a]	0.40	14
IL-6 ($pg.mL^{-1}$)	1.66 ± 2.26 [a] *	0.93 ± 1.29 [a]	0.28	2.41
TNFα ($pg.mL^{-1}$)	3.31 ± 5.42 [a]	2.56 ± 2.40 [a]	0.14	3.59
IL-1β ($pg.mL^{-1}$)	2.50 ± 9.99 [a] *†	0.00 ± 2.35 [a]	0.24	1.00
CRP ($mg.L^{-1}$)	1.62 ± 2.40 [a] *	0.77 ± 0.88 [a]	0.13	3.35
IgG ($mg.dL^{-1}$)	1649 ± 865 [a] *	835 ± 695 [a]	0.49	1696

WBC = white blood cell count, PLT = platelet count, MPV = mean platelet volume, NEUT = neutrophil count, LYMPH = lymphocyte, MONO = monocyte, EOS = eosinophil, BASO = basophil, NLR = neutrophil lymphocyte ratio, cTnT = cardiac troponin T, IL-6 = interleukin-6, TNFα = tumour necrosis factor alpha, IL-1β = interleukin-1 beta, CRP = C reactive protein, IgG = immunoglobulin G.

Table 3 Association between monthly wear limit (≤ or > 9 wears per month) and participants IL-6, IL-1β, CRP and IgG above or below upper reference levels. Odds ratios based on likelihood of those completing >9 wears per month exhibiting haematological levels above reference limits. * denotes significant association ($p < 0.05$).

	Chi squared	*p* value	Odds Ratio (95% CI)
IL-6	11.981	0.002 *	6.27 (2.04 – 19.3)
IL-1β	7.784	0.004 *	7.00 (1.51 – 32.51)
CRP	21.023	0.001 *	12.43 (3.57 – 43.22)
IgG	14.563	0.001 *	7.55 (2.41 – 23.61)

IL-6 = interleukin-6, IL-1β = interleukin-1 beta, CRP = C reactive protein, IgG = immunoglobulin G

Table 4 Association between haematological group (above or below reference limits) with the presence of symptoms of ill health. Odds ratios based on the likelihood of those above reference limits exhibiting symptoms of ill health. * denotes significant association ($p < 0.05$).

	Chi squared	*p* value	Odds Ratio (95% CI)
IL-6	11.695	0.002 *	5.63 (1.96 – 16.20)
IL-1β	5.131	0.040 *	3.67 (1.13 – 11.90)
CRP	10.507	0.004 *	6.05 (1.86 – 19.72)
IgG	13.792	0.001 *	6.45 (2.24 – 18.58)

IL-6 = interleukin-6, IL-1β = interleukin-1 beta, CRP = C reactive protein, IgG = immunoglobulin G

FIGURES

Figure 1 IgG (A), IL-1β (B), IL6 (C) and CRP (D) plotted against monthly wear number. Green zone represents the "healthy" range for each variable, as identified in Table 2. Vertical grey dashed line represents the 9 exposure limit. IL-6 = interleukin-6, CRP = C reactive protein, IL-1β = interleukin-1 beta, IgG = immunoglobulin G.